THE REACTION OF VARIOUS BACTERIA UPON AESCULIN AGAR.*

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GREAT attention has been given to the bacteriological analyses of "open waters" to determine their value as potable waters. Exhaustive determinations have been made of the bacterial flora of various rivers and lakes. The common result of most of these observations has been that the colon bacillus is present to a greater or lesser degree in every "open water." However, tho different streams may contain colon bacilli, it is found that the numbers which are present in a definite quantity of the water vary; but when a certain stream is analyzed at intermittent periods for a year or more, it is found that the number of bacilli present in that water is fairly constant for the same period in the year, except when a new source of pollution has entered the stream within the viable distance of the colon bacillus. If this source of pollution is continuous, a new colon standard is developed for that stream.

It is universally accepted that B. coli is a fair indicator of pollution in any water, and that when the "normal" coli content has been determined in a water, any unusual fluctuation, arising above this normal, is indicative of a new pollution.

Hence, too, a bacteriological analysis of water mainly concerns itself in the determination of the presence of colon bacilli, besides estimating the total bacterial count per cubic centimeter of water, and the number of liquefiers of gelatin present. When occasion demands, definite pathogenic organisms are searched for in the water.

As, therefore, the routine water analysis is so closely associated with colon bacilli, many investigators have devised special methods to determine the colon bacillus in definite quantities of water. Most of these methods have been, in truth, devices for separating the colon bacillus from the many other organisms present in the water, and some workers have obtained a definite biological reaction on certain media to serve as an indicator of the presence of this bacillus.

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It has been found that the colon bacillus is more resistant to certain chemical substances than are other bacteria, so that media containing these substances will propagate this bacillus better than others. Such media have been prepared with phenol and hydrochloric acid (Parietti), crystal violet (Conradi-Drigalski), malachite green (Löffler), bile (McConkey), caffein, and other substances. By the use of these media most of the bacteria other than those belonging to the colon-typhoid are group inhibited in their growth. From such cultures then the remaining bacteria may be isolated and their characters determined. Some workers deem it quite sufficient to inoculate dextrose fermentation tubes from growths in the above inhibitory media, and to determine (by the production or non-production of gas in the proportion of $H:CO_2 = 2:1$) the presence or absence of the colon group. This presumptive test is used extensively in America. In the English laboratories the use of the bile-salt media has found preference (McConkey).

It is scarcely necessary to point out that most of the presumptive tests have their shortcomings in one way or another. We have been particularly struck with the inaccuracy of the gas determination for the colon bacillus. We have noted that when colon bacilli were present in mixt cultures in the carbohydrate broth either they produced no gas whatever, or that the amount of gas was very much reduced, and in altered proportions.

Recently Harrison and van der Leck[1] have introduced still another medium for the determination of colon bacilli and excretal bacteria in water or milk. They have found that when the glucoside (aesculin) is present with iron citrate in the culture medium, the colonies of colon bacilli are black and have a black halo around them. These black colonies are readily distinguishable against a white background. They have found that B. lactis aerogenes, lactose-fermenting yeasts, and some molds give the same blackening of the media about the colonies as do colon bacilli. As, however, the yeasts and molds are readily distinguished in their colonies and are seldom found in water, they may be disregarded. In some of the experiments Harrison and van der Leck found that the colonies of B. lactis aerogenes could be distinguished from those of the colon bacilli. They point out

[1] *Centralbl. f. Bakt.*, Abt. II, 1909, 22, p. 547; *Proc. Roy. Soc. Can.*, 1908, S. III, 2, p. 105.

that as the B. lactis aerogenes may be regarded as an excretal form of organism, it is of value to recognize its presence. The authors state that some 40 species or varieties of bacteria and yeasts have been grown in media containing aesculin with negative results.

The authors have prepared a bile-salt aesculin broth and an aesculin agar which they recommend for the routine examination of water. In using the former medium the presence of colon bacilli is indicated by the black coloration of the fluid, and they consider this a better presumptive test than the use of the neutral red bile-salt broth.

The authors have found that in the analysis of water, the blackened bile-salt aesculin broth has invariably yielded colon bacilli. In the analyses of 60 samples of water, they have found the aesculin bile-salt test correct in 100 per cent.

In the following experiments we have undertaken the examination of many strains of colon bacilli, which have been isolated and fully determined as to their characters, besides testing the action of other bacteria on the aesculin media. Most of the bacteria tested were obtained from patients in the Royal Victoria Hospital. These bacteria had been isolated from time to time, and were retested as to their purity on several occasions.

In determining the action of the bacteria on aesculin media, we have used the formula as given by Harrison and van der Leck. The method of preparing the agar is as follows:

1 or 2 gm. of Witte's peptone.
0.5 gm. sodium taurocholate (commercial).
0.1 gm. aesculin.
0.05 gm. iron citrate (ferric).
100 c.c. tap water.

After steaming 15 to 30 minutes the medium is filtered and sterilized. For aesculin agar 1.5 per cent agar is used, being dissolved in part of the water, after which the other ingredients are added, the medium brought to boiling, filtered, tubed, and sterilized.

Plates were poured with this medium, and after hardening, the different organisms were planted on the surface, or deep colonies were made. The plates were incubated at 37° C. and were examined every 24 hours for a period of two weeks.

In most instances in which a positive result was obtained, the dis-

coloration of the medium occurred within 24 hours. In a few instances, however, the positive result was delayed for several days. One of the colon strains gave a positive result on the second day. Another colon strain was positive only on the third day, while a strain of B. mucosus remained negative until the fifth day, when a slight blackening of the medium occurred, which increased for several days. We have found that there is a certain variation in the positive tests. The typical positive tests in which the colonies are black with a black halo around them, as described by Harrison and van der Leck, do not constantly occur. Four colon strains we found gave only a faint brownish discoloration of the medium, immediately about the colony, while the colony itself was of a greyish-brown color. Again other colon colonies gave a reddish-brown discoloration of the medium without definite blackening of either the colony or the medium. One strain of proteus, which gave a positive result, first developed a yellow change in the medium, which later showed a greyish halo around the outer edge of the discoloration.

On account of the variable types of the color reactions which were obtained on the medium, we have included in the "positive tests" all reactions which in any way discolored the medium. This discoloration ranged, as above noted, from a yellowish-brown to a distinct black. The tests referred to as negative were those in which no color was present in either the colony or the medium. In this way we have tested 110 organisms of different strains, and from various sources with the following result:

	Positive Test on Aesculin Agar	Negative Test on Aesculin Agar		Positive Test on Aesculin Agar	Negative Test on Aesculin Agar
B. coli communior	3	10	B. iliacus	1	2
B. coli communis	10	6	B. cloacae	1	4
B. typhosus	0	19	B. subcloacae	1	0
B. typhosus-like organism	1	0	B. proteus vulgaris	1	9
B. paratyphosus B	0	13	B. proteus plebeius (Ford)	0	1
B. mucosus	3	1	B. pylori (Ford)	0	1
B. duodenale (Ford)	1	2	B. xerosis	0	1
B. lactis aerogenes	3	1	B. subtilis	0	1
B. pseudodysenteriae (Muller)	1	1	B. diphtheriae	0	3
B. dysenteriae (Flexner)	0	1	Staph. aureus	0	2
B. acidiformans	0	1	Staph. albus	0	3
B. alkaligenes	0	2			

It will be seen from the above table that most variable results were obtained from the various organisms. B. coli communior gave only

three positive tests in 13 strains, while B. coli communis gave 10 positive reactions in 16 strains. The positive tests obtained with the B. coli communis and communior were far from being uniform, and the color reactions varied from those producing no color at all, others producing a faint greyish-brown, to those showing red-brown, brown, or black color. These color reactions were evident either in the colonies themselves, or in the surrounding medium, or in both. The majority of the colon strains which gave a color reaction showed the dark punctate center in the colony with a black halo surrounding the colony, as described by Harrison and van der Leck.

Among our stock of 19 typhoid strains none was found to give any color reaction on aesculin agar. We have, however, an organism which was isolated from the gall-bladder of a typhoid subject, and which on media gave all the reactions of B. typhosus. At the time of isolation, this organism was tested with a known typhoid serum but gave a negative result. Subsequently at intervals of about a month this organism has been tested with various typhoid sera and has never agglutinated in dilutions higher than 1.25. Except for the agglutination reaction, this bacillus has all the features of B. typhosus. This bacillus was found to give a positive reaction on aesculin and the reaction was positive only on the seventh day, no reaction showing in the six days previously. The medium about the colony was discolored a faint brown which increased to a darker brown after several days. The reaction here obtained, altho not typically positive, as described by Harrison and van der Leck for certain colon strains, was nevertheless equal in intensity to that obtained with several colon organisms tested by us, and noted as positive in the above table.

Again, among four B. lactis aerogenes strains, we had one which remained negative in its reaction on aesculin agar after 14 days.

Summarizing our results, we found that in 62 different strains of 13 types of intestinal bacteria of man, 22 gave a positive reaction on an aesculin, while 40 were negative. In other words, the majority of the excretal type of bacteria, which were examined by us, and which had their known origin from man, gave a negative result when tested on the above medium.

In our hands this medium has not proved satisfactory for the

isolation and determination of B. coli and other human excretal bacteria.

NOTE.—Since going to press Harrison and van der Leck have in a recent article (*Centralbl. f. Bact.*, 1909, 51, p. 607) described some modification in the preparation of the media. The amount of iron citrate has been increased to 0.1 per cent, and they insist that the acidity of the media should be 0.6 per cent acid. The media which we have used in our experiments was of this acidity, but contained a smaller quantity of iron citrate. The authors point out that with an acidity lower than 0.6 per cent the results are not uniform. It is found too that the color of the colonies is dependent directly upon the amount of citrate and acid present.